ENCAPSULATION OF VIRUSES

Jerrold L. Anderson and S. David Butz

National Cash Register Company
Dayton, Ohio
Contract AF 29(601)-6344

TECHNICAL REPORT NO. WL-TR-64-167
August 1965

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Research and Technology Division
Air Force Systems Command
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FOREWORD

This report was prepared by the Capsular Research and Product Development Department of the National Cash Register Company, Dayton,Ohio under USAF Contract No. 29(601)-6344. Program Element was 6.14.45.01.4. The contract was initiated under Project No. 8803, "Encapsulation of Viruses." The work was administered under the direction of the Biophysics Branch, Air Force Weapons Laboratory, Lt Albert Ichiki, project engineer, (WLRB-2).

The report covers work conducted from March, 1964 through October, 1964. The report was submitted 9 August 1965.

National Cash Register Company personnel contributing to the research effort were:

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The aid of other personnel in the Capsular Research and Product Development Department and technical assistance of supporting departments is gratefully acknowledged.

This report has been reviewed and is approved.

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ABSTRACT

Research efforts were directed toward development of a method of encapsulating viruses in spheres containing a predictable phage titer to be used in biological dosimetry experiments. Initial studies demonstrated the ability of the bacteriophage to undergo lyophilization and encapsulation yielding a product of adequate viability. The Butarez-Toluene-Ethylcellulose encapsulation system proved satisfactory for producing spheroidal virus-ethylcellulose capsules of twenty-five to fifty microns diameter, the mean diameter being forty-two microns. Viability determinations yielded a phage titer of 1.7×10^{11} phages per gram of capsules; hence, a capsule of average diameter possessed a theoretical titer of 8×10^{3} phages. Simulated end-use tests proved photographic developer chemicals, such as could be used for the processing of virus-bearing nuclear emulsions, to be nontoxic to the encapsulated phages. A modification in the scope of the program directed the concluding work to the preparation of capsules less than fifteen microns in diameter. Samples of virus-ethylcellulose capsules were submitted to the Air Force.

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1. INTRODUCTION

The Air Force Weapons Laboratory proposed to formulate a biological dosimeter for the purpose of determining the cell damaging potential of the highly ionizing components of cosmic radiation at high altitudes. It is believed that biologically effective radiation dosages encountered at increasing altitudes can be determined by exposing simple organisms to the space radiation via high altitude vehicles. The infectivity of the organisms upon recovery should provide an indication as to the amount of energy absorbed by the biological material in the thin down of the heavy nuclei.

The biological dosimeter will consist of bacteriophage particles incorporated into a nuclear emulsion film. The viruses must be contained in some type of minute package so that, upon recovery of the film, they can be located and extracted for remaining infectivity determinations. A capsular container not only would contain a specific quantity of virus, but would also afford a degree of protection of the virus from the environment, i.e., protection from emulsion and developer chemicals, etc.

Upon entering a nuclear film emulsion, a cosmic ray leaves a "track" which appears as a streak on the developed film. If virus were contained in spherical capsules smaller than the cross section of the cosmic ray track, the exposed capsules could be manipulated from the path of the track and analyzed with respect to remaining infectivity. The comparison of the percentage of infective phages remaining in the selected capsule with an inactivation curve prepared by irradiating virus-containing capsules with 250 KVP X-ray would indicate the radiation dose absorbed by the particular virus-containing capsule.

Under Air Force Contract No. AF 29(601)-5233, the National Cash Register Company investigated the concept of microencapsulating the bacteriophage T1 using gelatin, primarily, as the protective coating material for the phage. The project demonstrated certain capabilities of the microencapsulation process, but was generally found to be inadequate for the satisfactory preparation of encapsulated phages applicable to dosimetry experiments.

Following the termination of the initial project, the National Cash Register Company further developed its capabilities in the area of encapsulated biologicals, primarily by utilizing ethylcellulose as a coating material. Hence, the current program involved the adaptation of the ethylcellulose microencapsulation process to the preparation of bacteriophage for dosimetry experimentation. The project was conducted March 1, 1964 through October 31, 1964.

2. RESEARCH ACTIVITIES

2.1 Basic Encapsulation Studies

Initial studies were directed toward ascertaining the ability of the bacteriophage to undergo lyophilization and encapsulation without incurring excessive loss of viability. Preliminary experiments were conducted using a mixture of bacterial viruses T1Hr, T1++, and ϕ_{II} in aqueous suspension, containing 1.3% solids and 2×10^9 virus particles per milliliter of stock solution. Fifty grams of finely divided humus were mixed with 450 milliliters of the phage suspension and the slurry was lyophilized. The dried product was encapsulated with ethylcellulose by applying the Butarez-Toluene-Ethylcellulose encapsulation system, a coacervation technique which is carried out at ambient temperature. The system proved feasible and further encapsulation experiments were made, without regard to particle size or shape, using lyophilized phages both with and without absorbents. Samples of the encapsulated products were submitted to the Biophysics Branch who determined the phage viability by quantitative plate studies.

TABLE I. Viability of Encapsulated Sorbed-Virus

Sample	Sorbent	Theoretical Count	Actual Count
No. 1324	(Control)	2×10^9 phages/ml.	1.21×10^9 phages/ml.
HR-26	Attapulgite Clay	2×10^{10} phages/gm.	3.7×10^7 phages/gm.
HR-27	No Additive	1×10^{11} phages/gm.	9.6×10^{10} phages/gm.
HR-28	Carbon Black	2×10^9 phages/gm.	1.6×10^7 phages/gm.
Humus	Humus	2×10^{10} phages/gm.	1.22×10^9 phages/gm.

It was concluded that phages could be (1) encapsulated by the Butarez-Toluene-Ethylcellulose system in high concentration and (2) retained in the biologically active state both with and without sorbents. It was noted also that the nonsorbed phage, Batch No. HR-27, yielded the highest total phage count and most nearly approached the theoretical concentration. Therefore, ensuing experimentation was limited to encapsulation of nonsorbed lyophilized phage solids by the Butarez-Toluene-Ethylcellulose system.

2.2 Capsule Size and Shape

The work statement of the contract directed research to the preparation of spherical capsules having a mean diameter between twenty-five and fifty microns with a coefficient of variation less than, or equal to, twenty-five as calculated from the expression,

$$C = \frac{S}{d} \times 100 \leqslant 25$$

where C is the coefficient of variation, S is one standard deviation (or σ), and d is the mean particle diameter.

Attapulgite clay of small particle size was used as a model internal phase in the first experiments to determine the variables affecting capsule size and shape and to establish the optimum values for the variables. Factors first considered include type of encapsulation vessel, placement of baffles, type of stirrer, and speed of agitation. The best appearing clay-ethylcellulose capsules were made in a variable speed Waring Blendor with a high-shear blade.

Lyophilized phage solids, ground to less than forty-four microns particle size, were first encapsulated using a standard method of agitation, i.e., stirring at a moderate rate with a turbine type stirrer in a large glass beaker. The product was rinsed thoroughly with petroleum distillate and air dried. The resultant particles, Batch No. HR-30, were irregular in size and shape (see Figures 1 and 2).

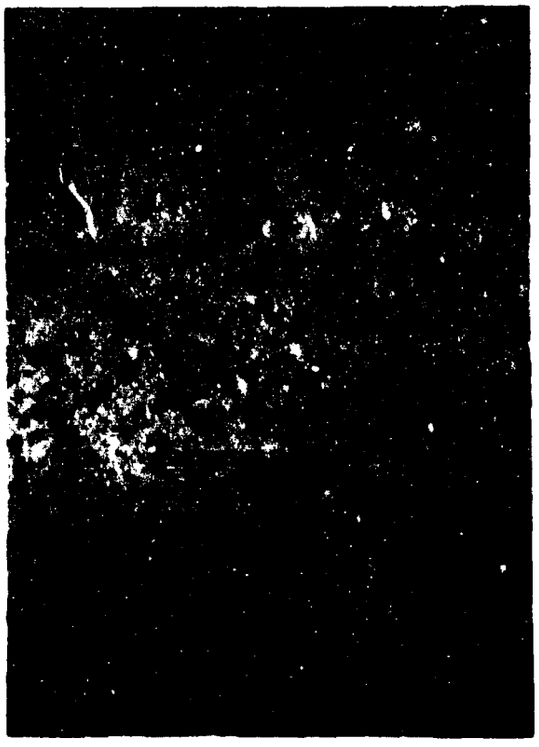

Figure 1. Photomicrograph of Batch No. HR-30, 100X

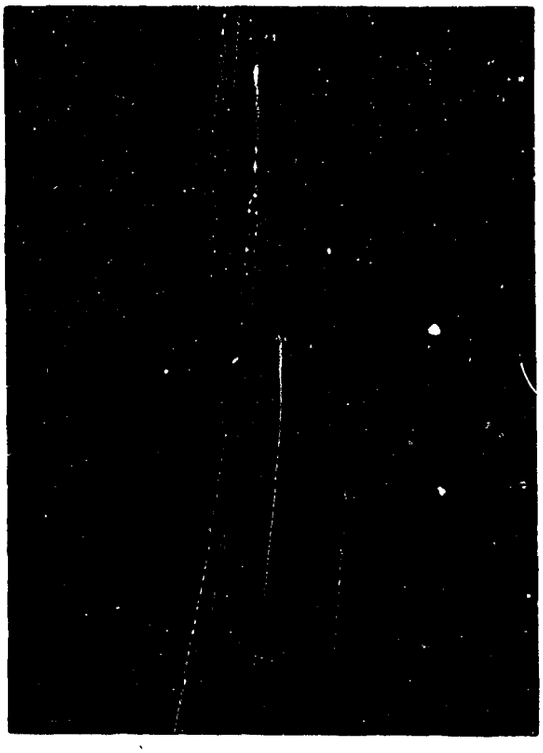

Figure 2. Photomicrograph of Batch No. HR-30, 200X

A second batch of phage particles, Batch No. HR-31, was encapsulated in the Waring Blendor with the speed reduced to a moderate rate by way of a variable powerstat. The resulting small particles (Figures 3 and 4) approached spheres in configuration.

The dried capsules were sieved to less than fifty-three microns diameter (270 mesh). Micromerograph analysis yielded an average particle diameter of 35.3 microns and a coefficient of variation of 26.1 (Figure 5).

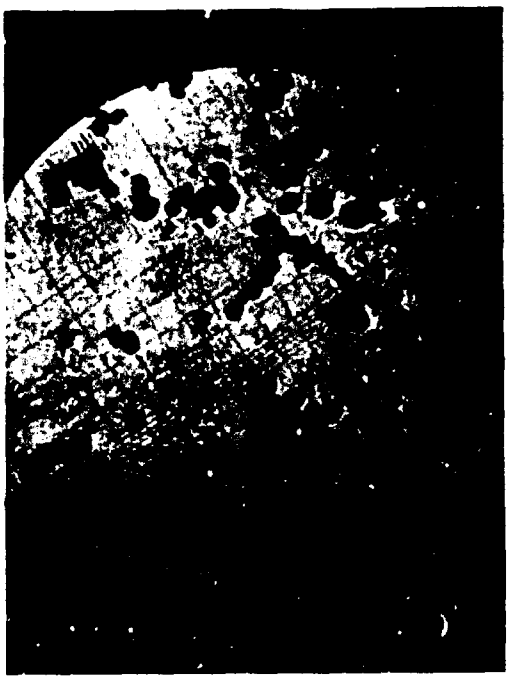

Figure 3. Photomicrograph of Batch No. HR-31, 100X

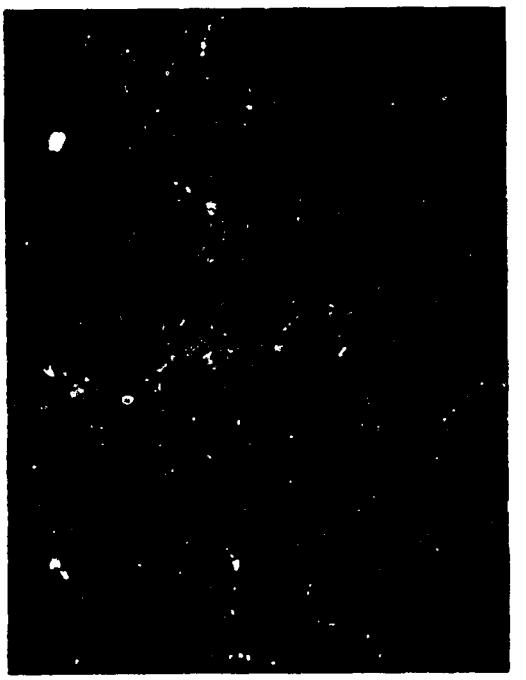

Figure 4. Photomicrograph of Batch No. HR-31, 200X

Attempts to demonstrate the reproducibility of the encapsulation system yielded capsules inferior to Batch No. HR-31 with respect to sphericity and uniformity. Therefore, subsequent experimentation was directed toward learning the optimum values for several variables in the encapsulation system in an attempt to develop a method of consistently producing capsules of acceptable shape and uniformity. A number of variables, such as (1) type of encapsulation vessel, (2) means of agitation, (3) phase ratio (phage: ethylcellulose), (4) amount of Butarez added, (5) rate of Butarez addition, (6) temperature of the system, and (7) ethylcellulose concentration, were postulated to have an effect, directly or indirectly, on the nature of the resultant capsules.

A total of twenty batches of attapulgite clay model material and eight batches of virus solids were encapsulated while systematically altering the several variables. These tests confirmed the reproducibility

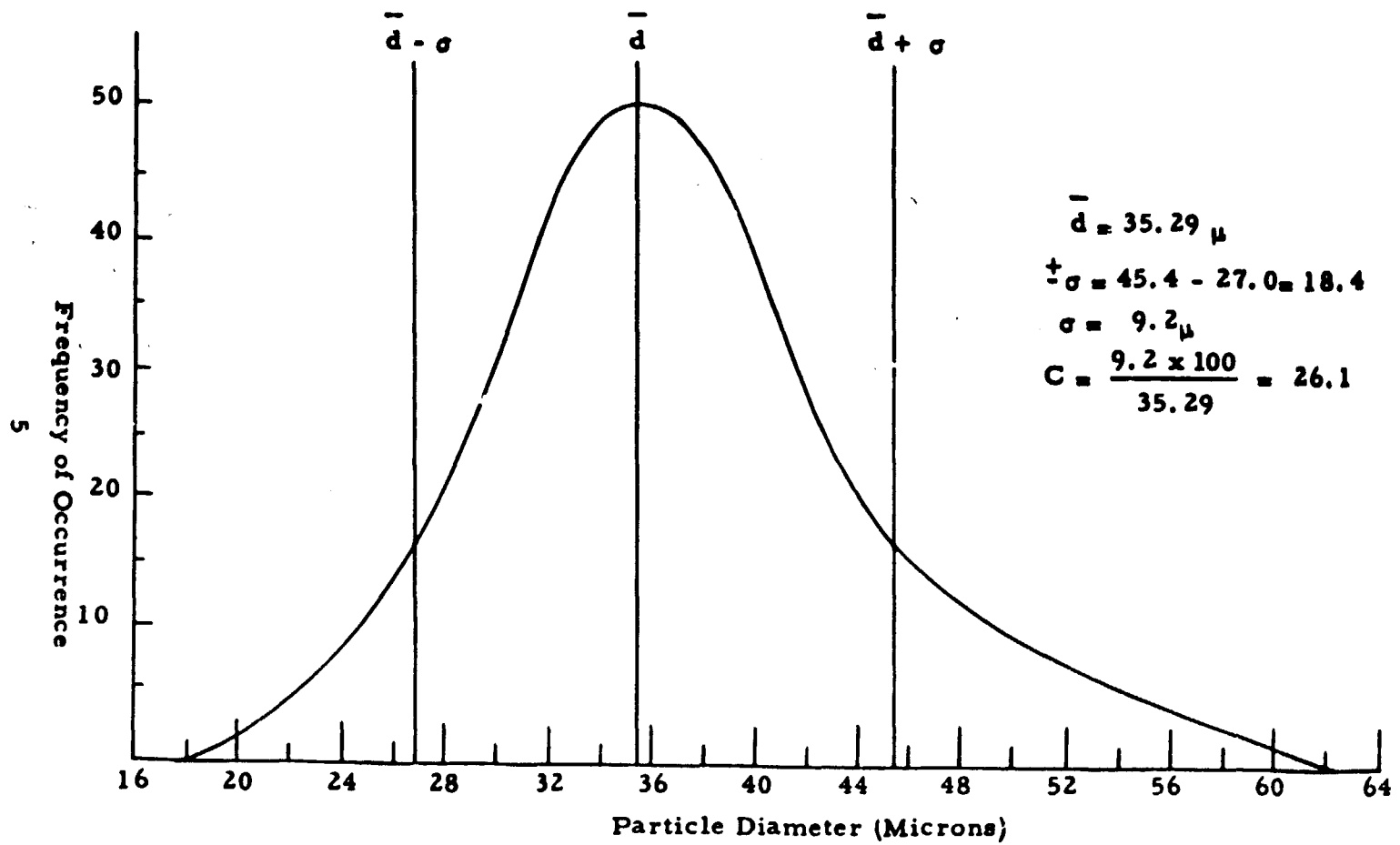

Figure 5, Size Distribution of Batch No. HR-31

of particle size and uniformity but even the best samples were less spherical than Batch No. HR-31. Batch No. SDB-19 (Figures 6 and 7) was one of the better samples prepared in this series of encapsulation trials.

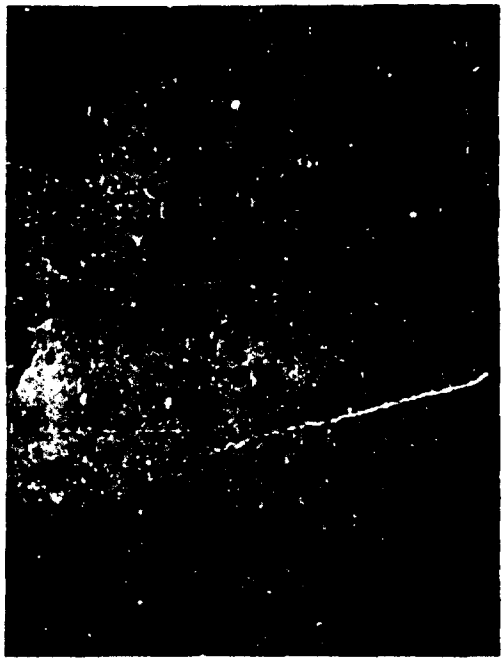

Figure 6, Photomicrograph of Batch No. SDB-19, 100X

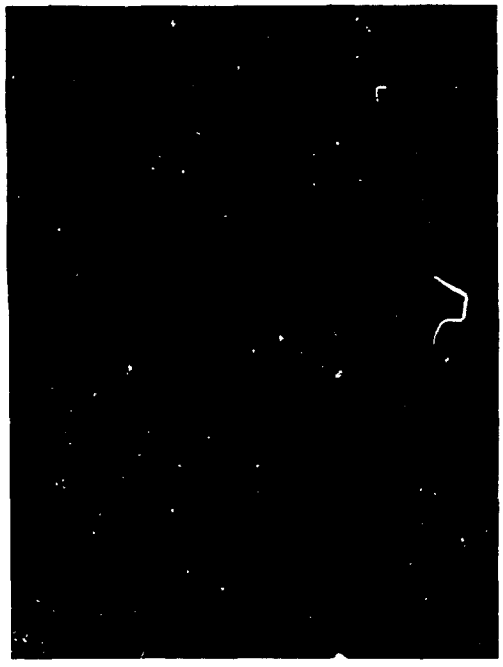

Figure 7, Photomicrograph of Batch No. SDB-19 200X

By micromerograph analysis the average diameter of particles in Batch No. SDB-19 was found to be 39.8 microns, with a coefficient of variation of 18.8 (see Figure 8).

2.3 Viability of Encapsulated Phage

2.3.1 Preliminary Viability Determinations

First attempts to determine the viability of encapsulated phages by the plaque assay procedure[1] yielded inconclusive results. Some of the procedural problems encountered were (1) incomplete dissolution of the capsule wall leading to nonhomogeneous phage dispersion, (2) non-representative sampling, (3) dispersion media incapable of sustaining the viable phages, and (4) exposure to materials detrimental to either the virus or the host bacteria. The development of a satisfactory method of dispersing the encapsulated virus is described in Table II.

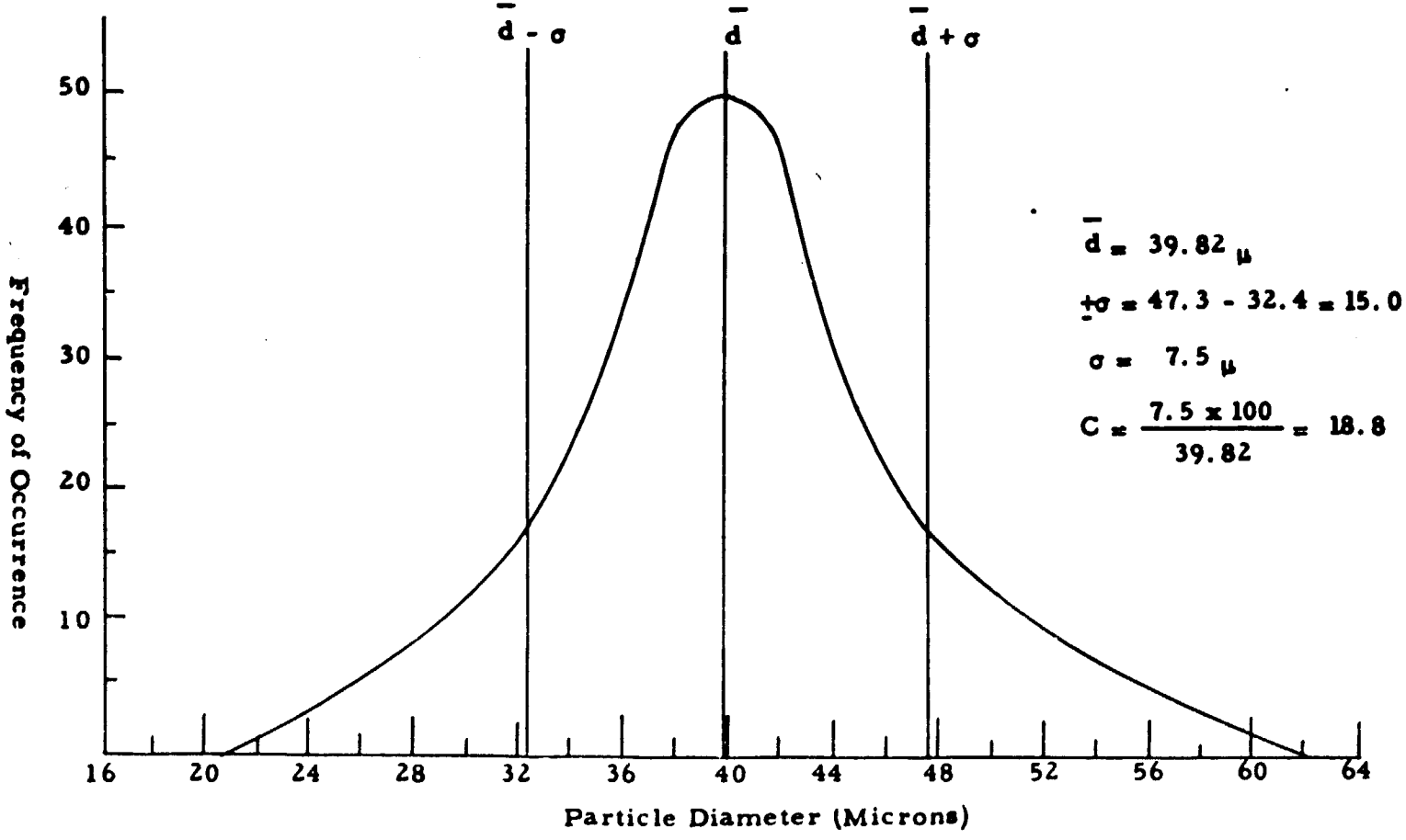

Figure 8, Size Distribution of Batch No. SDB-19

TABLE II. Dispersion Studies With 10 mg. Virus-Ethylcellulose Capsules

Trial No.	Solvents	Results	Conclusions
1	5 ml H_2O +5 ml Acetone	Capsules formed a clump which turned white and floated.	It appeared that the ethylcellulose shells remained, some of the solids were extracted.
2	1 ml H_2O + 5 ml Acetone	Formed clumps, turned white and floated.	Result appeared similar to (1)
3	5 ml H_2O + 1 ml Acetone	Capsules were separated, turned white, did not settle out with centrifugation.	Apparently the virus solids were extracted from the ethylcellulose shell, which floated.
4	5 ml cold 30% Ethanol	Solids were suspended but settled out with centrifugation.	Virus solids were insoluble.
5	5 ml H_2O + 5 ml Toluene	Capsules dispersed but the solvents separated.	Solvents were immiscible, phage not well dispersed.
6	Placed in 2 ml Acetone, then added 5 ml H_2O	Capsules separated in acetone, yellow solids settled out. Dispersed completely in H_2O.	Wall material apparently dissolved in acetone, virus solids dispersed in H_2O, homogeneous dispersion results.
7	Placed capsules in 4 ml Acetone, added 6 ml H_2O	Capsule walls dissolved in acetone, virus solids dispersed in H_2O, long time centrifugation yielded nothing.	Suggest this method for future viability study serial dilutions.

The technique for serial dilution and plating of virus samples for plaque assay is illustrated in Figure 9.

By implementation of the foregoing techniques, quantitatively reproducible viability determinations were made on samples of liquid phage dispersions, lyophilized phage solids, and virus-ethylcellulose capsules. Figure 10 shows the growth of a lawn of the bacteria Escherichia coli strain B on which the bacteriophage formed plaques. The holes in the lawn, a few millimeters in diameter, surround each virus.

Viability determinations by the plaque assay procedure were made on several samples of phage capsules during the course of the research work and compared with the theoretical count. The results appear in Table III.

TABLE III. Viability of Encapsulated Phages

Sample	Theoretical Count	Actual Count
SDB-19	1.3×10^{12} phages/gm.	1.46×10^{10} phages/gm.
SDB-38	1.3×10^{12} phages/gm.	2.23×10^{10} phages/gm.
HR-75	2.04×10^{12} phages/gm.	1.7×10^{11} phages/gm.
SDB-81	1.57×10^{12} phages/gm.	7.8×10^{10} phages/gm.
SDB-82	1.57×10^{12} phages/gm.	1.1×10^{11} phages/gm.
SDB-83	1.57×10^{12} phages/gm.	1.0×10^{11} phages/gm.
SDB-84	1.57×10^{12} phages/gm.	1.2×10^{11} phages/gm.

2.3.2 Single Capsule Studies

The intended application for the virus capsules dictates that the number of phages in a capsule of known diameter be predictable. The calculation of theoretical phage count in a capsule of given diameter is demonstrated below.

Variable	Value Assigned
Mean capsule diameter	35 microns (0.0035 cm.)
Specific gravity of capsules	1.40
Phase ratio	0.667
Phage concentration of stock solution	1.30×10^{10} phages/ml.
Solids content of stock solution	1.3%

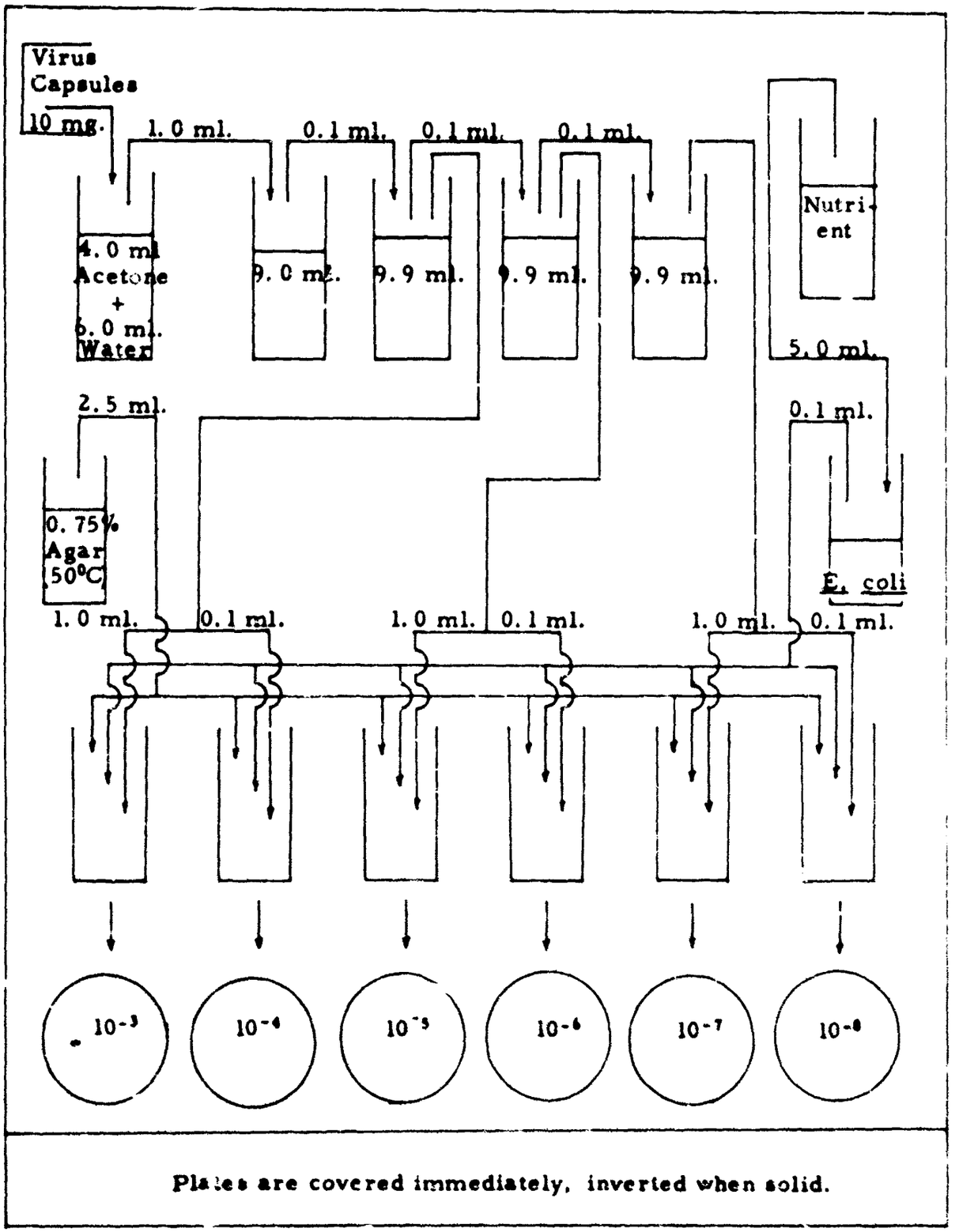

Figure 9, Serial Dilution for Plaque Assay

Calculations

A. Titer of one gram solids

$n = (1.30 \times 10^{10}$ phages/ml.) / (0.0130 gm. solids/ml.)

$n = 1.0 \times 10^{12}$ phages/gm.

B. Volume of one capsule

$V = (\frac{4}{3})\ (3.14)\ (0.00177$ cm.$)^3$

$V = 2.30 \times 10^{-8}$ cc./capsule

C. Weight of one capsule

$W = (1.40$ gm./cc.$)\ (2.30 \times 10^{-8}$ gm./capsule$)\ (0.667)$

$W = 2.16 \times 10^{-8}$ gm./capsule

D. Number of phages per capsule

$N = (2.16 \times 10^{-8}$ gm./capsule$)\ (1.00 \times 10^{12}$ phages/gm.)

$N = 2.16 \times 10^{4}$ phages per capsule

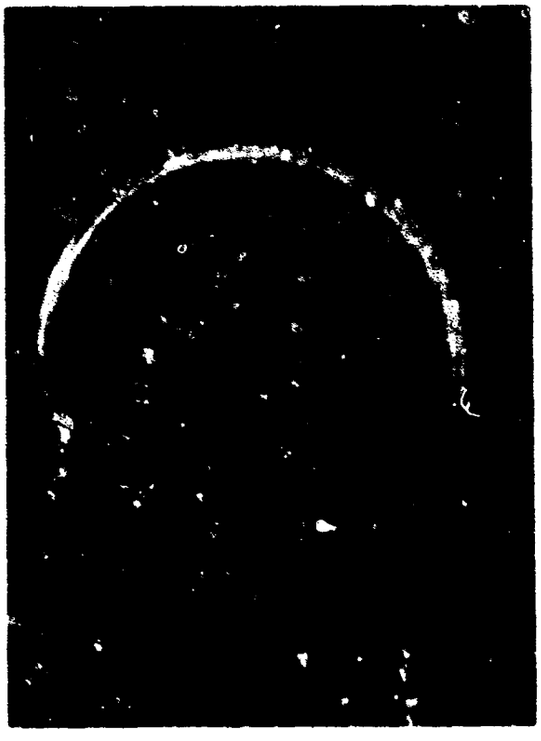

Figure 10. Lysed Bacterial Lawn

The theoretical phage count is graphed versus capsule diameter in Figure 11. It is noted that capsules in the twenty-five to fifty micron range theoretically contain from 8000 to 60,000 virus per capsule, a viability level believed to be adequate for the end-use application.

Preliminary single capsule viability counts were made on Batch No. HR-75. A few individual capsules were lifted from a microscope slide by means of a moist drawn-glass capillary as in Figure 12.

The capsules, whose diameters were estimated by optical microscopy, were dispersed as previously described and analyzed by plaque assay. The resulting single capsule counts are compared to the theoretical values in Figure 13. It is believed that a more accurate method of capsule diameter measurement and representative sampling of the capsule population would produce more precise and satisfactory results thereby increasing the degree of predictability.

2.4 Stability Testing

2.4.1 Capsule Wall Permeability

The permeation of phages across the ethylcellulose wall with respect to time elapsed in water suspension was investigated. Small samples of capsules from Batches SDB-19 and SDB-38 were suspended in water. The extraction medium was sampled and mounted for quantitative viability determinations at predetermined time intervals. The results are summarized in Table IV.

The fact that the viability count of the extraction fluid after a twenty-four hour suspension exceeds the count after longer times suggests that the phages have a limited viability in water. The increase of phage inactivation with elapsed time is attributed, at least in part, to osmotic shock[2].

A number of twenty-four hour viability tests were conducted with virus suspensions in various media to select a fluid capable of sustaining the phages in a viable state for long periods of time. A solution of 0.9 molar sodium chloride in nutrient broth proved satisfactory; therefore, this solution was used as the suspension medium in subsequent wall permeation tests.

To learn the rate of release of phages from the capsule structure, samples of virus-ethylcellulose capsules were suspended in nutrient solutions which were sampled for plaque assay at progressive time intervals. Table V indicates the results.

Phage Concentration = 1 x 10^{12} Phages/Gram

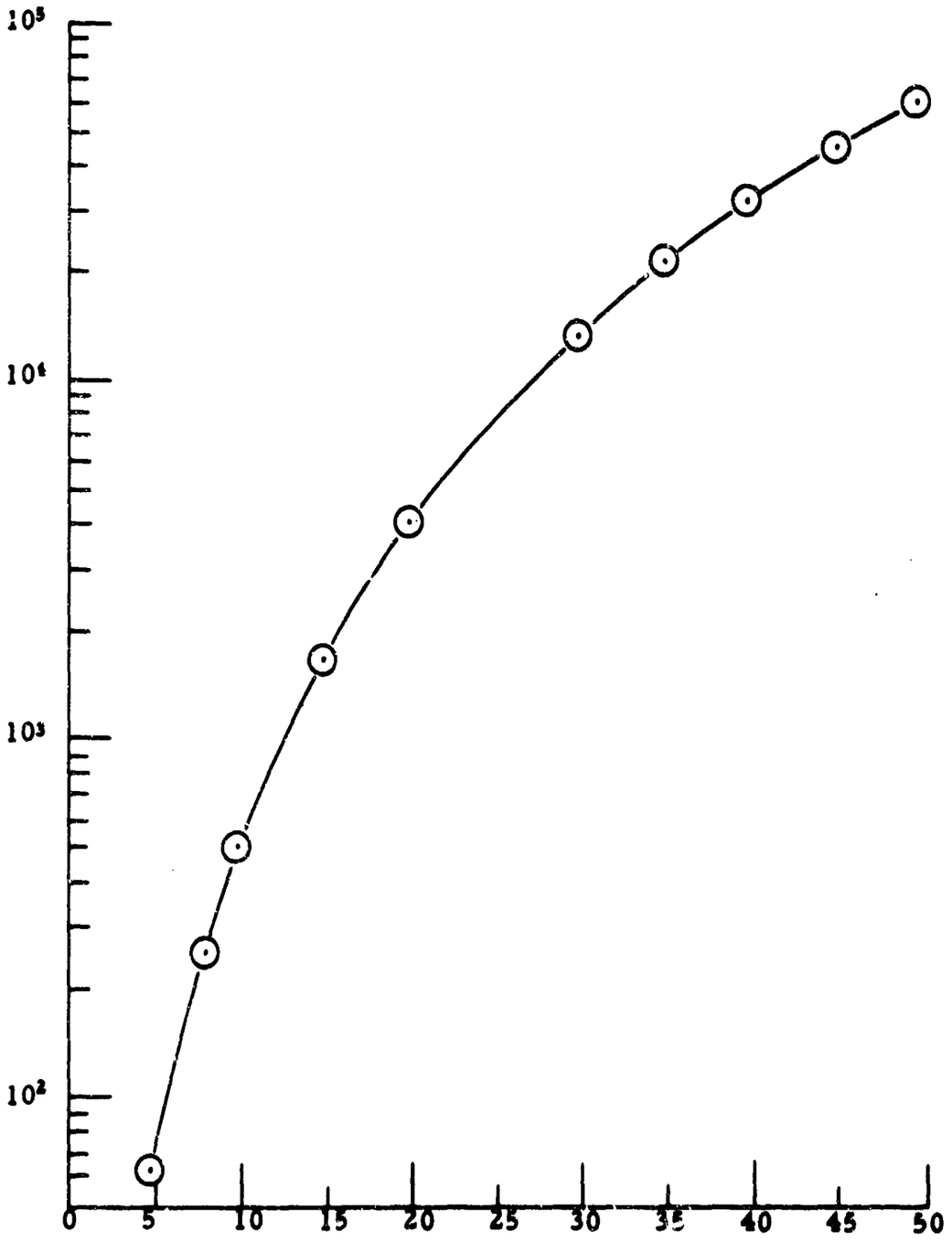

Figure 11. Theoretical Single Capsule Viabilities

TABLE IV. Phage-Capsule Permeation in Water Suspension

Batch No.	Sample	Dilution	Plaques	Phages per ml. of Extraction Fluid	Phages extracted per gm. starting material
SDB-19	Dispersed capsules	10^{-5}	101	1.01×10^{7}	
		10^{-5}	163	1.63×10^{7}	
		10^{-5}	173	1.73×10^{7}	1.46×10^{10}
SDB-19	24 Hour extraction	10^{-5}	99	$.99 \times 10^{7}$	
		10^{-5}	109	1.09×10^{7}	
		10^{-5}	103	1.03×10^{7}	9.9×10^{9}
SDB-19	74 Hour extraction	10^{-3}	138	1.38×10^{5}	
		10^{-3}	173	1.73×10^{5}	
		10^{-3}	226	2.26×10^{5}	1.68×10^{8}
SDB-38	Dispersed capsules	10^{-5}	251	2.51×10^{7}	
		10^{-5}	314	3.14×10^{7}	
		10^{-5}	203	2.03×10^{7}	2.23×10^{10}
SDB-38	24 Hour extraction	10^{-4}	274	2.74×10^{6}	
		10^{-4}	293	2.93×10^{6}	
		10^{-4}	270	2.70×10^{6}	2.79×10^{9}
SDB-38	120 Hour extraction	10^{-4}	1	1×10^{4}	
		10^{-4}	2	2×10^{4}	
		10^{-4}	2	2×10^{4}	1.5×10^{7}

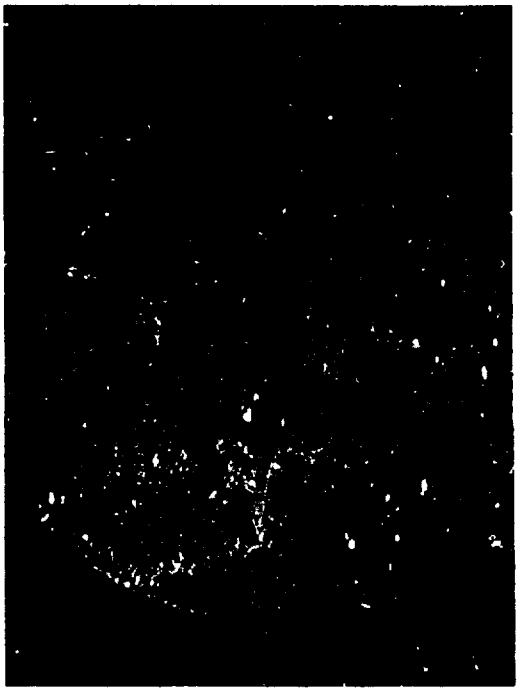

Figure 12. Manipulation of Single Capsules, 100X

TABLE V. Capsule Wall Permeability

Suspension Time	Phages per milliliter of Nutrient	Phages Released per Gram Capsules	Percent of Original Released
1 hour	3.2×10^5	2.9×10^8	7.6%
4 hours	3.2×10^5	3.0×10^8	7.9%
24 hours	5.8×10^5	5.7×10^8	15.0%

It had previously been suggested that a photographic developing process of relatively short duration would be a less rigorous test of the ability of the encapsulated virus to withstand the developer chemicals than the usual long-duration nuclear emulsion developing process. [3,4] It is believed that if the capsule-bearing nuclear emulsion is cast in a film less

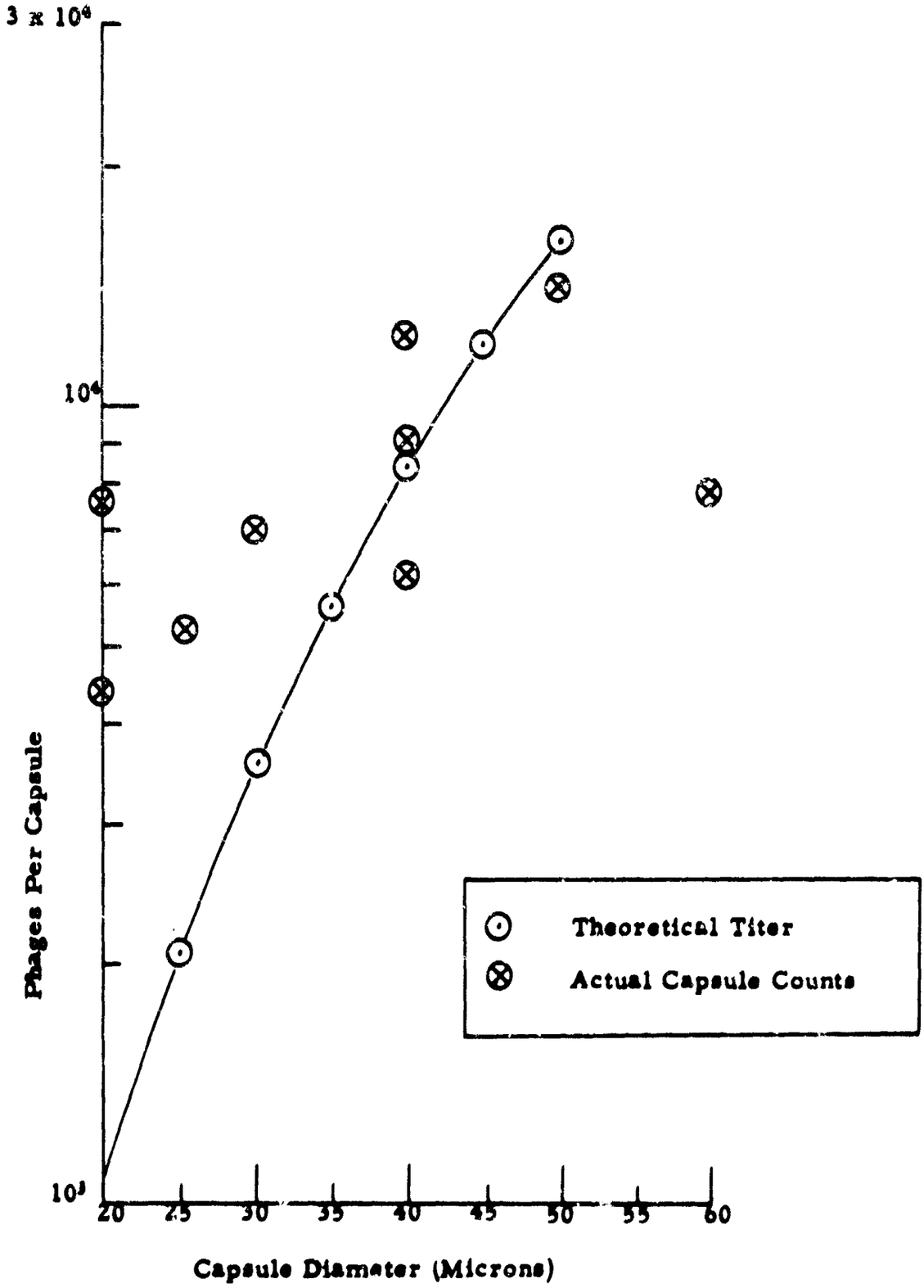

Figure 13. Single Capsule Viability Counts, Batch No. HR-75

than 300 microns in thickness, a standard X-ray developing process can be employed. The entire processing time would total less than one hour. Consideration of the data in Table V leads to the conclusion that the ethylcellulose wall is adequate to contain the virus during photographic (such as X-ray) processing without excessive loss. Also, it is assumed that phage permeation will be further reduced in the gelled emulsion.

2.4.2 Toxicity of Developer Chemicals

Simulated end-use studies were conducted to determine if photographic developing chemicals are toxic to the phages within the capsules. Samples of virus-ethylcellulose capsules were suspended in individual photographic processing chemicals with results as summarized in Table VI.

TABLE VI. Toxicity of Developer Chemicals to Virus

Developer Constituent	Suspension Time	Viability (phages/ml.) Test Sample	Control*	% Remaining Active
Developer, Eastman	15 min.	1×10^7	1×10^7	100%
Short stop, 25% Acetic Acid	1 min.	0	1×10^7	0%
Short stop, .5% Acetic Acid	1 min.	1×10^7	1×10^7	100%
Fixer, 30% Sodium Thiosulfate	30 min.	1×10^7	1×10^7	100%

* Control = Phage capsules treated similarly, less chemicals.

Samples of virus-ethylcellulose capsules were taken through the entire simulated photographic developing process. The steps were as follows: fifteen minutes suspension in developer, neutralization with acetic acid, followed by a thirty minute suspension in fixer. Throughout the process no appreciable loss of viability was incurred.

2.4.3 Dry Capsule Stability

Two samples of encapsulated virus were analyzed with respect to viable phage concentration over a period exceeding three months (Figure 14).

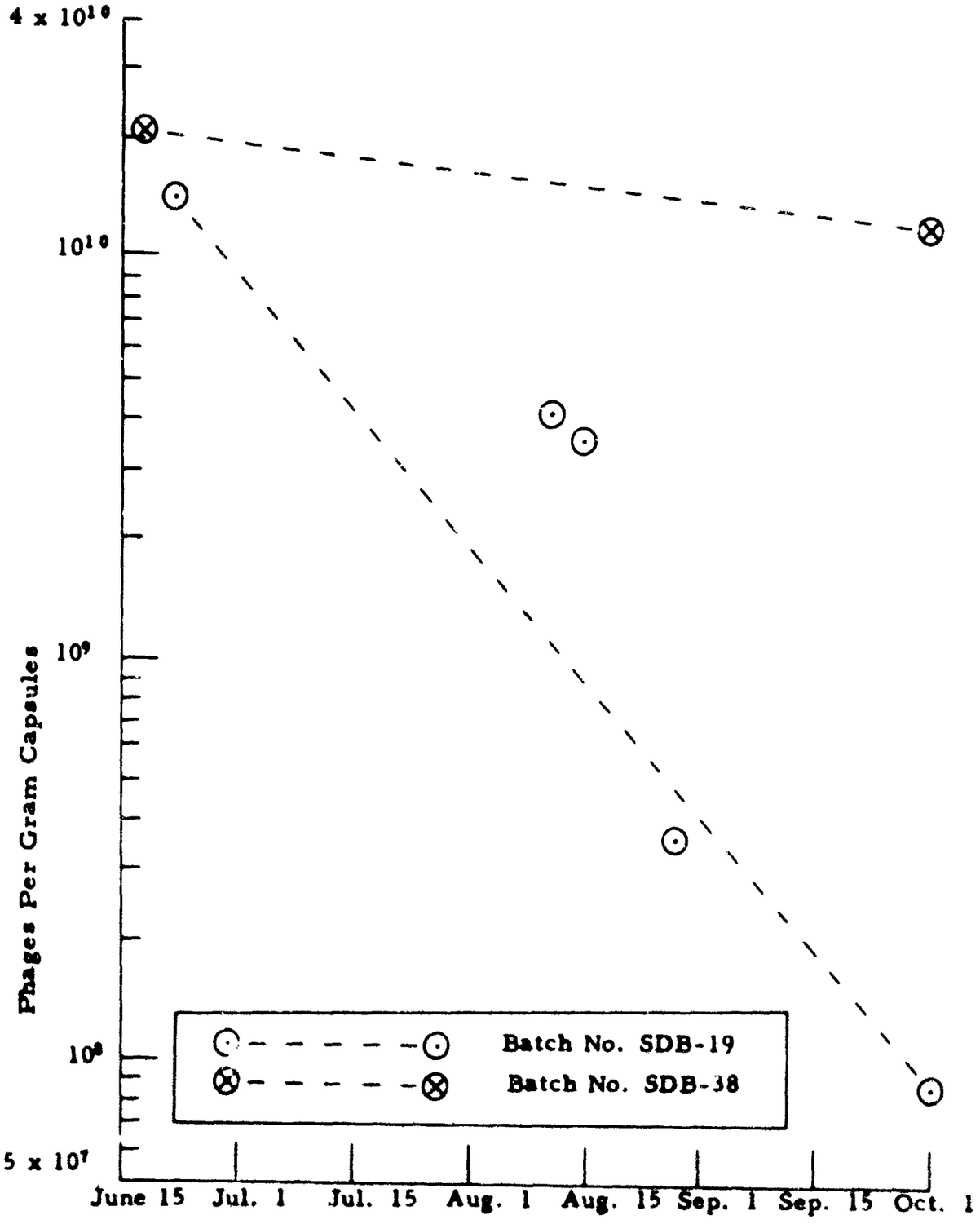

Figure 14. Dry Capsule Stability at 25°C

The viability of Batch No. SDB-19 was determined shortly after preparation. The capsules were stored in an open container, exposed to the laboratory atmosphere. Due to the hygroscopic nature of the capsules, the material became quite damp during the test period. Periodic plaque assay determinations indicated that the viability diminished somewhat steadily over the test period.

Batch No. SDB-38 was analyzed by plaque assay immediately after preparation of the capsules and again after more than three months had elapsed. During the intervening time the sample was sealed in an air-tight container at ambient temperature wher it remained dry and free-flowing.

The results of this stability test indicate that, under proper storage conditions, virus-ethylcellulose capsules can be retained for periods in excess of three months without appreciable loss of viability.

2.5 Delivery of Material

Having established a method of reproducibly preparing spheroidal virus-ethylcellulose capsules of twenty-five to fifty microns diameter (Section 2.2), a large batch was prepared in ten gram increments. The oversized capsules and aggregates were removed from the incremental batches by selective sieving and discarded. The capsules falling between twenty-five and fifty microns were combined to form Batch No. HR-75, (Figures 15 and 16), of which 100 grams were delivered to the Biophysics Branch. Figure 17 describes the size distribution as determined by micromerograph analysis. Data pertinent to the sample include:

Particle Size

Average diameter = 42.7 microns
Coefficient of variation = 26.3

Viability

1.7×10^{11} phages per gram of capsules
4000 to 15,000 phages per capsule (Theoretical count based on phages/gm. and capsule size data).

The specific gravity of Batch No. HR-75 virus capsules was determined, using magnaflux oil in a liquid pycnometer. In Table VII the experimental value is compared to the value as calculated from the densities of the components.

TABLE VII. Specific Gravity of Virus Capsules

Trial	Sp. gr. of Virus Solids	Sp. gr. of Ethylcellulose	Calculated Sp. gr. of Capsules	Experimental Sp. gr. of Cap.
1	1.59	-	-	1.47
2	1.62	-	-	1.46
Average	1.61	1.14	1.45	1.47

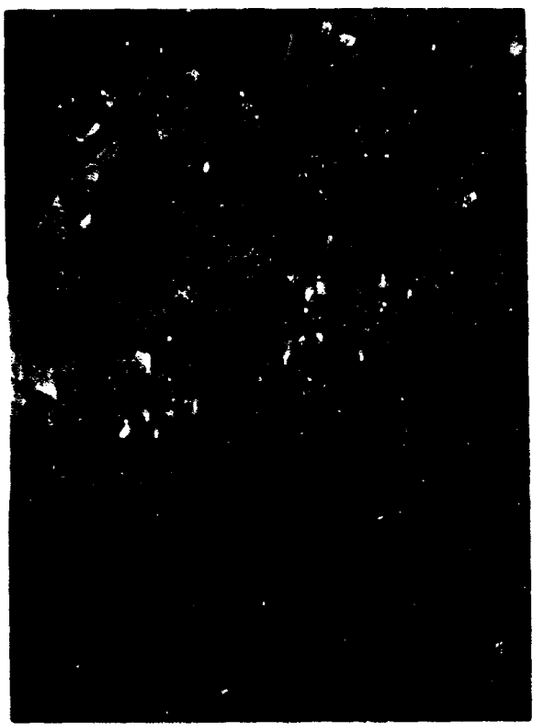

Figure 15. Photomicrograph of Batch No. HR-75, 100X

Figure 16. Photomicrograph of Batch No. HR-75, 430X

2.6 Preliminary Small Capsule Studies

The feasibility of preparing virus-ethylcellulose capsules having an average diameter of from five to fifteen microns was investigated.

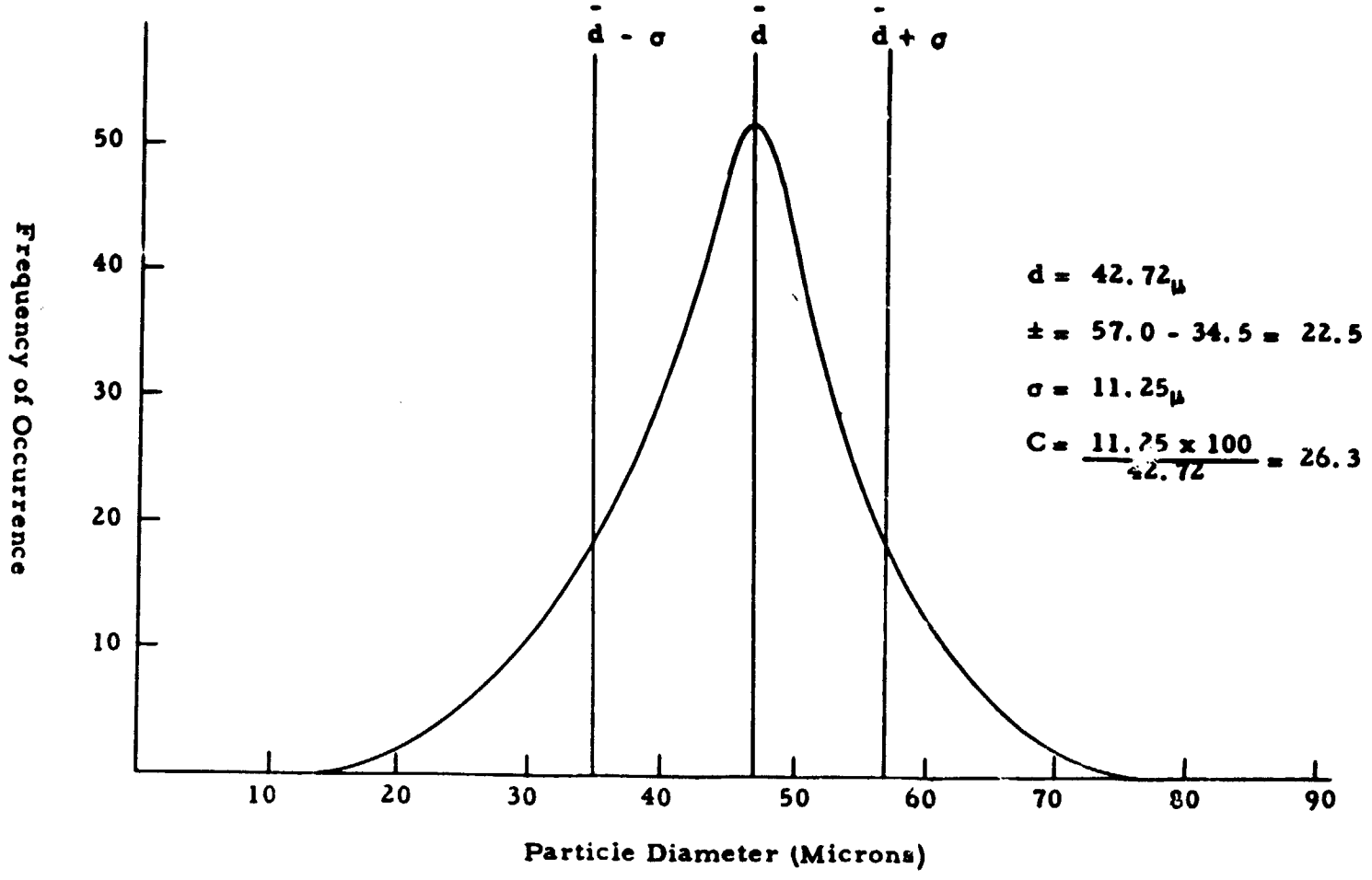

Figure 17. Size Distribution of Batch No. HR-75

Several experimental batches of virus were encapsulated in an attempt to determine the feasibility of preparing phage capsules of appropriate size, viz., five to fifteen microns in diameter. The first attempt, designated Batch No. HR-76, was prepared by reprocessing large (i.e., twenty-five to fifty microns diameter) virus capsules. The wall material was dissolved off the virus with toluene in a Waring Blendor and the phage was re-encapsulated at a higher rate of agitation. Microscopic observation (Figures 18 and 19) indicated that the preparation yielded an average capsule diameter greater than fifteen microns. As attested by Figure 20, it was observed that some of the particles of virus solids were larger than the desired capsule size.

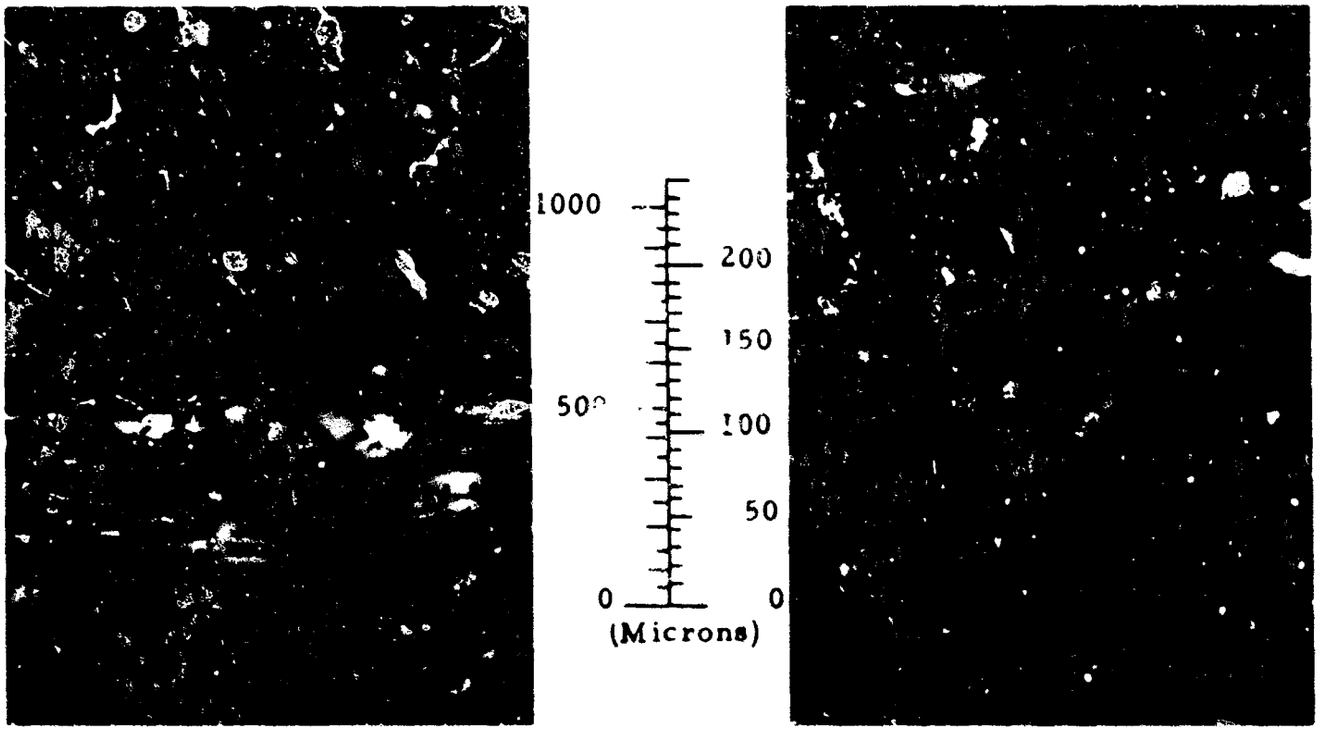

Figure 18. Photomicrograph of Batch No. HR-76, 100X

Figure 19. Photomicrograph of Batch No. HR-76, 430X

In further attempts to produce small capsules, a process included milling of the phage solids before encapsulation. Eight grams of lyophilized phage solids were dispersed in one hundred grams of toluene and milled in a small porcelain ball-mill for one hour. The material was then encapsulated in the Waring Blendor at high speed. When observed in dispersion, the capsules, designated Batch No. SDB-80, appeared to be of excellent shape and size, i.e., spheres less than fifteen microns in diameter (Figure 21). However, the particles aggregated on drying due to the presence of residual phase-inducing polymer (Figures 22 and 23). The long sedimentation time required to remove the very small particles from suspension necessitates more numerous washes to remove the polymer.

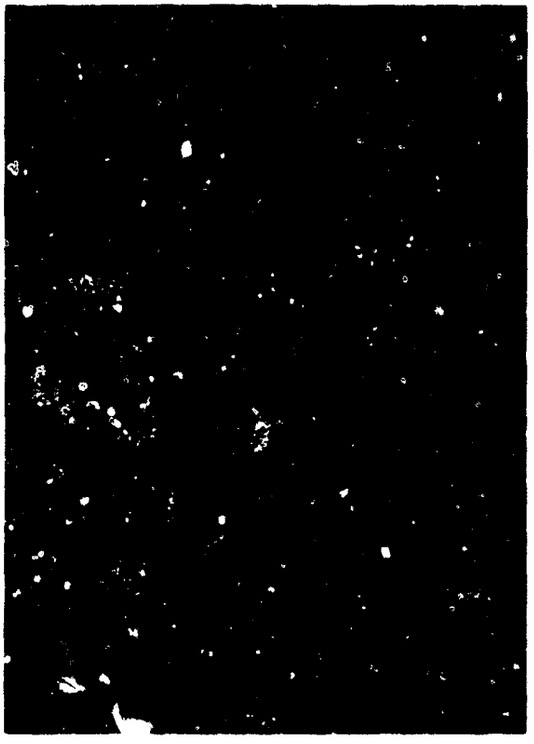

Figure 20. Photomicrograph of Batch No. HR-76, 1000X

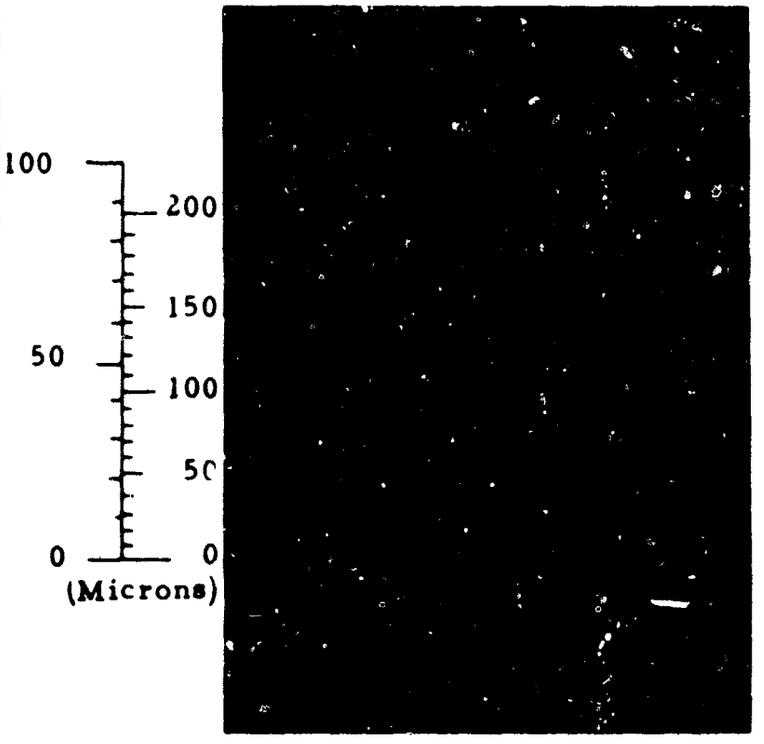

Figure 21. Photomicrograph of Batch No. SDB-80, 430X, Dispersed

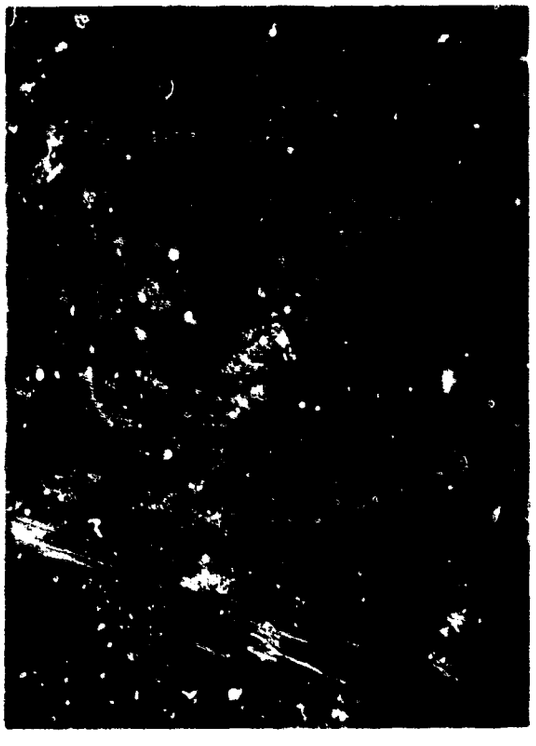

Figure 22. Photomicrograph of Batch No. SDB-80, 100X, Aggregated

Figure 23. Photomicrograph of Batch No. SDB-80, 430X, Aggregated

Batch No. SDB-81 was prepared similarly to the former batch with the exception that it was washed more thoroughly, effectively removing the excess polymer. Although oversize particles were removed by selective sedimentation and decantation, it is noted in Figures 24 and 25 that some large (greater than fifteen microns in diameter) particles remained. Batches SDB-82 SDB-83, and SDB-84, of which Figures 26 and 27 are representative, were prepared by the method previously described, demonstrating the reproducibility of the system. Although the classification of particles by sedimentation appeared quite efficient, a small fraction of oversize particles was retained in the samples. The phage viabilities of these batches, as summarized in Table III, indicate a high titer, viz., an average titer of 1×10^{10} phages per gram of capsules. However, the extremely low volume of a single capsule physically limits the virus concentration to less than one hundred phages per capsule (Figure 28). Size determinations on the small particle batches were approximated by optical microscopy because an inherent static charge caused aggregation to occur during attempted micromerographic analysis.

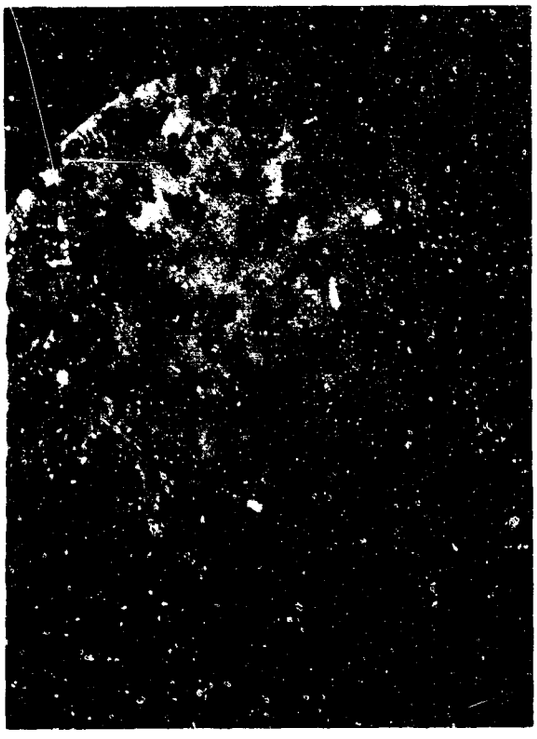

Figure 24. Photomicrograph of Batch No. SDB-81, 100X

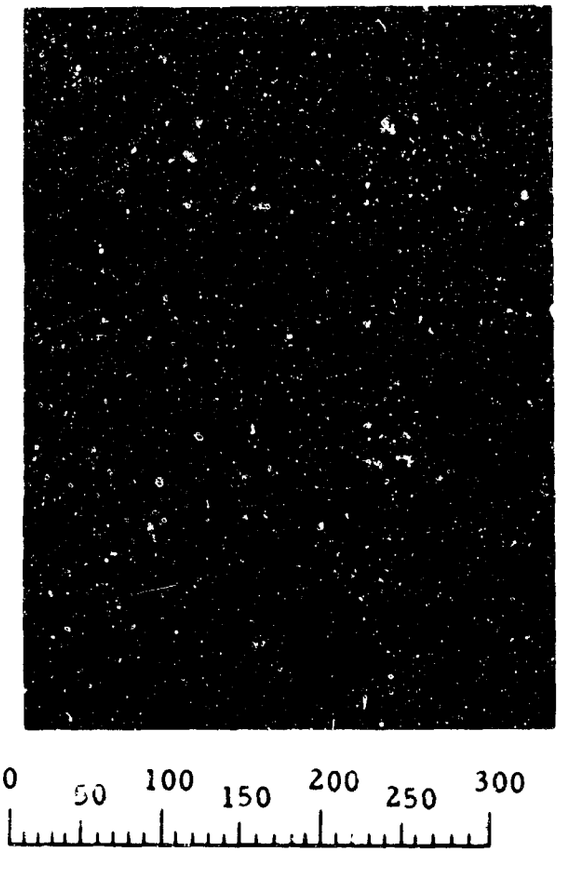

Figure 25. Photomicrograph of Batch No. SDB-81, 430X

Figure 25, Photomicrograph of Batch No. SDB-82, 100X

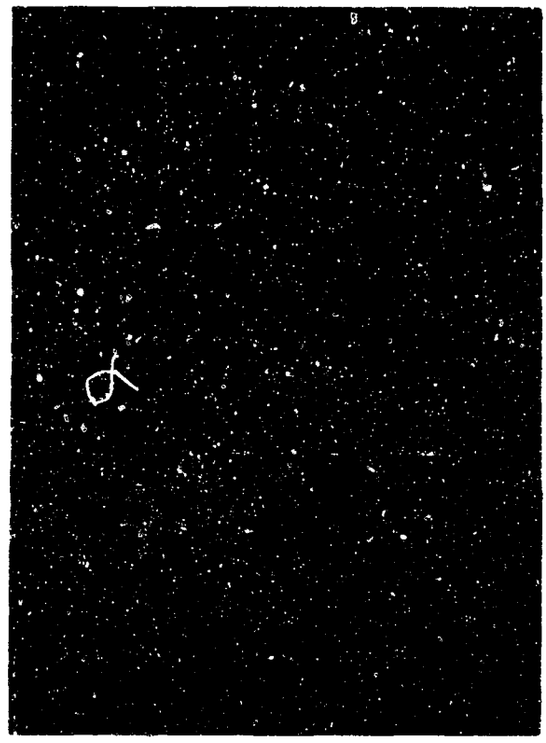

Figure 27, Photomicrograph of Batch No. SDB-82, 430X

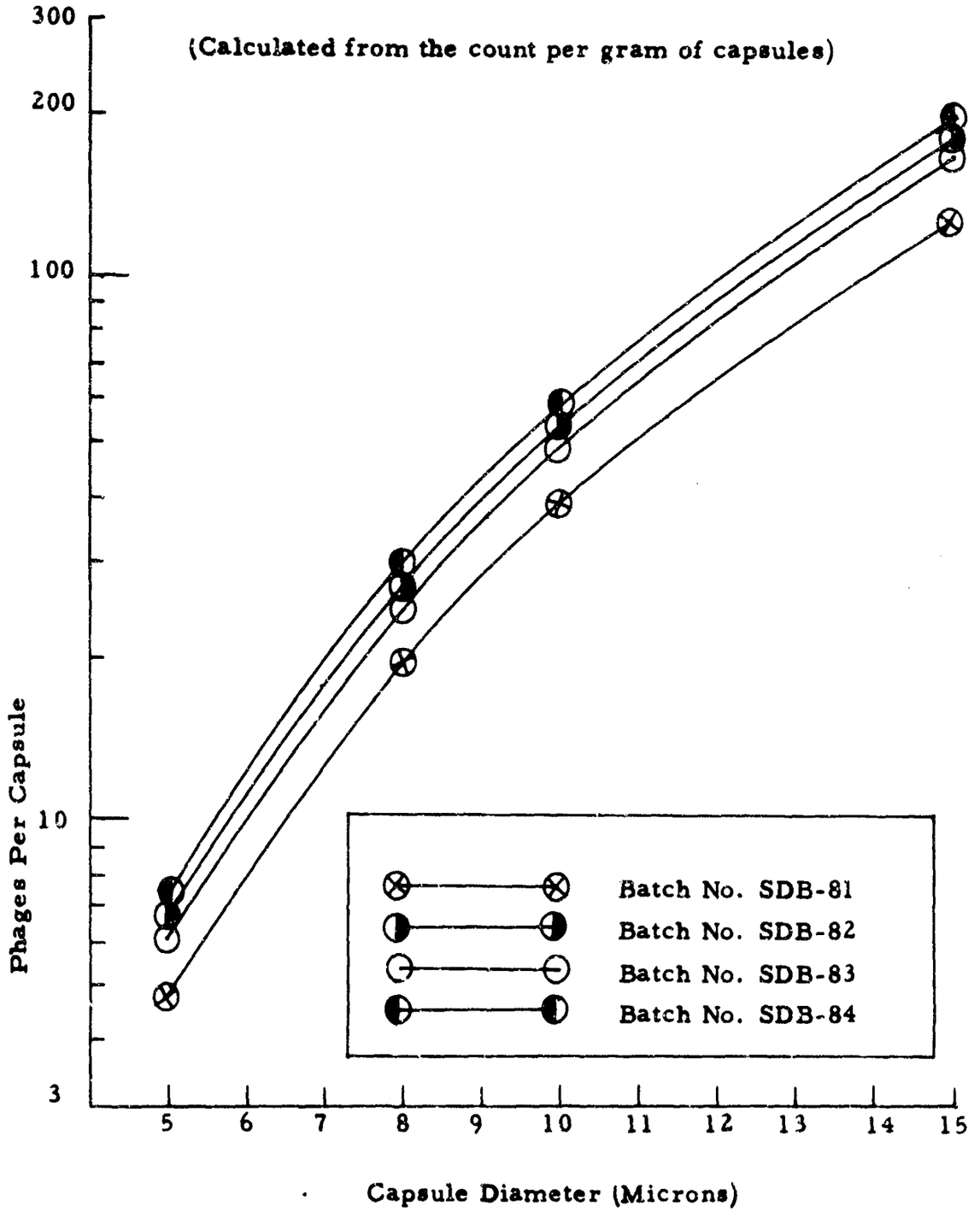

Figure 28, Theoretical Viabilities of Small Single Capsules

3. CONCLUSIONS

The experimental endeavors conducted during the program have led to the following conclusions.

1) Certain bacteriophages (T1Hr, T1++, and ϕ_{II}) can be lyophilized and subsequently encapsulated with ethylcellulose.

2) The encapsulated product, prepared from a stock aqueous phage dispersion containing 10^{11} phages per milliliter and 1.5% solids, will possess a phage titer of 10^{11} phages per gram of capsules.

3) The phages can be reproducibly encapsulated to yield spheroidal microcapsules having a mean diameter of twenty-five to fifty microns and a coefficient of variation ($C = \frac{S \times 100}{\bar{d}}$) of approximately twenty-five.

4) The number of phages in a single capsule should be in the range of 4000 to 16,000. This value is dependent upon the initial phage concentration of the stock aqueous dispersion and the capsule size, and therefore appears predictable.

5) The encapsulated phages are stable to the conditions of a simulated end-use; namely, the development of a silver halide nuclear emulsion (X-ray film).

6) The encapsulated phages can be stored under appropriate, dry conditions with no appreciable loss of viability resulting for at least three months.

7) Lyophilized bacteriophages can be ball-milled, encapsulated with ethylcellulose, and sized by sedimentation to produce a capsule product of five to fifteen microns in diameter.

8) The viability of the small capsules is equivalent, on a weight basis, to that which is obtained with the larger twenty-five to fifty micron capsules, i.e., about 10^{11} phages per gram of capsules. However, the capsule concentration of phages is limited to the range of approximately ten to two hundred phages per capsule as this value again is a function of the capsule size and starting concentration of the stock phage dispersion.

4. RECOMMENDATIONS

The results obtained during this contract have indicated the feasibility of using microencapsulated bacteriophages in the design of a cosmic thin down dosimeter. The preliminary small capsule studies conducted during the final stages of the program indicate that the preparation, per se, of capsules five to fifteen microns in diameter is not problematic. Hence, the problem becomes one of preparing capsules having an appropriate, predictable phage titer. A capsule concentration of 10^3 to 10^4 phages per capsule should be possible if a purified phage stock solution having a concentration of 10^{13} phages per milliliter could be obtained.

It has been reported that the purification of the phage media should be feasible. The predictability of the capsule phage content should again be a function of capsule size, and the determination of the degree of predictability will involve the development of a precise method for measuring the diameter of the individual five to fifteen micron capsules.

In summation, the following recommendations outline the remaining efforts which, it is believed, will result in the application of the encapsulated bacteriophage - cosmic dosimeter concept.

1) Prepare a purified phage stock dispersion such that after lyophilization the phage titer is not less than 10^{14} phages per gram of solids.

2) Prepare ethylcellulose-phage capsules spheroidal in shape and ranging from five to fifteen microns in diameter (Microsieving and/or sedimentation techniques should allow this size range to be readily attainable.)

3) Determine the relationship between capsule size and phage titer. This will involve the development and utilization of a precise measurement of the capsule diameters.

4) Test the capsules by simulating end-use applications. That is, place the phage capsules in a nuclear emulsion, expose and develop the emulsion, extract the capsules from the developed film, and determine the viability.

5. REFERENCES

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2. Adams, Mark H., "Bacteriophage," Interscience, 1959.

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4. Yagoda, Herman, "Radioactive Measurements with Nuclear Emulsions," Wiley, 1949.